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DURATION OF ACTIVITY OF THE MICROBIAL LARVICIDE VECTOLEX CG® (*BACILLUS SPHAERICUS*) IN ILLINOIS CATCH BASINS AND WASTE TIRES

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ABSTRACT. The duration of activity of a formulation of *Bacillus sphaericus*, VectoLex CG®, for control of *Culex* species was evaluated in 338 catch basins in Urbana, IL, and compared to Altosid® in 346 catch basins in Champaign, IL. The activity of VectoLex in car and truck waste tires was evaluated in a tire dump located in Pembroke Township, IL. In catch basins, 1 g of VectoLex per catch basin gave the same control as one Altosid briquet. Both larvicides were effective against *Culex* sp. in catch basins for 1 month, and the duration of control with VectoLex lasted 44 days in one catch basin. VectoLex was considerably cheaper to apply than Altosid briquets, at 0.64 cents per catch basin compared to 90.75 cents, respectively. However, the Altosid briquets were judged to be easier to apply from a vehicle than VectoLex granules. VectoLex (22.6 kg) was used to treat approximately 6,000 car and truck tires; some of the tires were in direct sunlight whereas others were shaded. *Aedes triseriatus* was the dominant species in these tires. Tires treated with VectoLex contained significantly fewer mosquitoes than control tires, and even 65 days after application, control tires were 16.7 times more likely to contain larvae. We conclude that VectoLex was effective when used in Illinois catch basins and tire dumps, and emphasize that it is more appropriate to base tire treatment rates on the total number of tires present than on a kilogram per hectare basis.

KEY WORDS *Bacillus sphaericus*, larvicide, catch basin, waste tires, VectoLex, Altosid

INTRODUCTION

VectoLex CG® (*Bacillus sphaericus* serotype H5a5b, strain 2362, Abbott Laboratories, North Chicago, IL) is a microbial larvicide formulated on 10–14 mesh corncob granules that is toxic when ingested by mosquitoes belonging to the genera *Aedes, Anopheles, Culex, Mansonia,* and *Psorophora* (Lacey 1990, Yap 1990, Groves and Meisch 1996, Mulla et al. 1997). Its larvicidal activity is due to 2 proteins of 41.9 and 52.4 kDa that act as a binary toxin (Davidson and Yousten 1990). The current label for VectoLex states that this product kills mosquitoes belonging to the genus *Culex* in water with a high organic content. Siegel and Novak (1997) conducted a pilot study that evaluated the efficacy of VectoLex in Illinois catch basins and waste tires. We reported that in catch basins the toxicity of VectoLex was comparable to Altosid® (Clarke Outdoor Spray, Inc., Roselle, Illinois) briquets and that in waste tires no pupae belonging to *Aedes triseriatus* (Say), *Culex pipiens* (L.), and *Culex restuans* (Theobald) were recovered as long as 67 days after treatment.

In this study, which was done in collaboration with the Champaign County Encephalitis Prevention Program (CUEPP) and the Kankakee Department of Public Health, we report the duration of control achieved by VectoLex when used as the sole larvicide in the catch basins of Urbana, IL. We compare these data to the duration of control achieved by Altosid in the catch basins of Champaign, IL. We also evaluate the efficacy of VectoLex used to treat part of a waste tire dump located in Pembroke Township, IL, and discuss our philosophy on the application rate necessary for achieving mosquito control in tires.

MATERIALS AND METHODS

Catch basin selection and dosage: In collaboration with the CUEPP, the catch basins in Urbana, IL, were treated with VectoLex CG and the catch basins in Champaign, IL, were treated with Altosid briquets. Catch basins holding water in Urbana neighborhoods where larvae were recovered received 1 g of VectoLex granules administered by a calibrated scoop. This dose was repeated at 1-month intervals throughout the summer. This dose was based on the study of Siegel and Novak (1997). Catch basins holding water in Champaign where larvae were recovered received one Altosid briquet; this dose was repeated at 1-month intervals in accordance with label instructions. All treated catch basins were marked with spray paint. No control catch basins were used in this study because our goal was to evaluate efficacy of VectoLex CG in an active control program. The majority of catch basins used in this study (>90%) were in proximity to trees.

Sampling: Neighborhoods in both cities were selected randomly and the marked catch basins were sampled at intervals of 14, 18, 21, 28, 30, 33, and 35 days after treatment in order to determine the duration of control. Catch basins were sampled by

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scooping contents using weighted strainers (Siegel and Novak 1997). A catch basin was scored as negative if no larvae or pupae were recovered after 3 scoops. If larvae were present in VectoLex-treated catch basins, the catch basins were scored as positive. Larvae and pupae recovered from Altosid-treated catch basins were brought back to the laboratory in water collected from the catch basin and reared in wax cups containing this water at 21°C. When adults emerged, the catch basin was scored as positive, otherwise the catch basin was scored as negative.

Persistence of VectoLex: A single catch basin in Champaign previously identified as a site favored by *Cx. restuans* and *Cx. pipiens* for oviposition (Siegel and Novak 1997) was treated with 1 g of VectoLex on June 17. This catch basin was sampled periodically and the date when it became positive was recorded.

Tire dump description: In collaboration with the Kankakee County Health Department and Illinois Environmental Protection Agency, a tire dump was selected for study in Pembroke Township, IL. A portion of this dump containing approximately 6,000 tires was used. Some of the tires were in direct sunlight without shade (<20%), whereas deciduous trees (oak and maple) shaded the remainder of the tires. All of the tires were within 10 m of the treeline. These tires were divided into 5 sections using flags; each section contained approximately 1,200 tires. Two sections contained car and truck tires, and the other 3 sections contained car tires. In all sections some of the tires were stacked in columns at least 10 tires high. After treatment, an adjacent group of car and truck tires, some of which were in full sunlight and others that were shaded, were used as controls.

Application: A dose of 22.68 kg of VectoLex CG was applied to the tires using a Stihl backpack applicator (Clarke Outdoor Spray, Inc., Roselle, Illinois). On a per-tire basis, this was 3.78 g. We assumed that 90% of the dose would miss the target (Siegel et al. 1996) and each tire would actually receive 378 mg, the same dose evaluated by Siegel and Novak (1997). The amount of granules broadcast within a study area depended on the size of tires (truck or car) and the type of stacking. The dosage rate per hectare throughout the pile was estimated by placing 23 buckets with a 27.94-cm-diameter opening in the tires and then weighing the granules trapped in the buckets after application.

Observation intervals: The tires were treated on July 15, 1997. Immediately before treatment, the percentage of tires containing larvae in each section was calculated by taking a single scoop from 100 tires, using a plastic cup (at least 360 ml water) and then pouring the water into an enamel pan. The presence or absence of larvae and pupae was recorded. The mosquitoes and larvae were then poured back into the tires. Tires in treated and control sections were sampled 14, 28, and 65 days after application, as described above, except that the sample was discarded rather than poured back into the tires. This was done to minimize agitation, which might resuspend the toxicant. Tires were marked with spray paint after sampling to avoid resampling at a later date. Larvae were collected and brought back to the laboratory for identification on the date of application and on day 65 after application. On day 14 after treatment, 518 treated tires and 60 control tires were sampled, on day 28 after treatment 520 treated tires and 103 control tires were sampled, and on day 65 after treatment 439 treated tires and 100 control tires were sampled. The odds ratio, a statistic used in epidemiology, was used to compare the likelihood of untreated tires containing mosquitoes compared to tires treated with VectoLex (Kelsey et al. 1986).

Table 1. Catch basin data for Champaign and Urbana, IL, July and August 1997.

Days after treatment	Negative catch basins	Positive catch basins	Dry catch basins
	Champaign; Altosid		
14	32	1	9
14	43	1	8
14	43	1	8
21	31	1	7
21	23	4	14
21	33	2	5
28	22	5	7
30	16	10	20
Total	243	25	78
	Urbana; VectoLex		
14	39	0	5
14	31	1	10
14	39	0	5
18	37	0	7
21	30	0	11
21	37	3	3
33	30	0	4
35	17	11	18
Total	260	15	63

RESULTS

Duration of control in catch basins

No significant difference was found in the duration of control achieved by Altosid and VectoLex, using contingency chi-square table analysis (Table 1). A total of 338 VectoLex-treated catch basins was sampled in Urbana, of which 63 (18.6%) were dry. Of the remaining 275, 15 (5.5%) were positive. More than 98% of these catch basins were negative as long as 33 days after treatment, but by 35 days after treatment only 35% of the catch basins were negative. The single catch basin monitored weekly was negative for 44 days after treatment. A total of 346 Altosid-treated catch basins was sampled in Champaign, of which 78 (22.5%) were dry. Of the remaining 268, 25 (9.3%) were positive (Table 1).

More than 93% of the Altosid catch basins were negative as long as 28 days after treatment, but by the 30th day only 61% of the catch basins were negative.

Catch basin treatment cost

In this study, VectoLex CG cost $6.39 per kilogram, and at the application rate of 1 g per catch basin, each treatment cost 0.64 cents. A briquet of Altosid cost 90.75 cents, which is a 141.8-fold difference in price. If we assume 3 treatments per season, the total cost per catch basin was 1.90 cents for VectoLex and was $2.83 for Altosid.

Ease of application in catch basins

The CUEPP personnel found it more difficult to apply VectoLex granules from a vehicle than Altosid briquets. When applications were made by foot, CUEPP personnel found VectoLex granules as easy to apply as Altosid.

Mosquito species in tires

When larvae were collected from the tires and brought back to the laboratory, *Ae. triseriatus* was the dominant mosquito species, and *Cx. restuans* and *Cx. pipiens* were the 2nd most prevalent species present. These results are typical for tires in central Illinois (Siegel et al. 1992, Siegel and Novak 1997).

Application rate per hectare

The average weight of granules per bucket (±SD) was 3.45 ± 2.98 g. Based on an area of 613.1 cm^2 per bucket, dosage ranged from 18.4 to 1,876.5 kg/ha, with an average of 562.7 kg/ha.

Duration of larvicidal activity in tires

Five hundred tires were counted before treatment and 451 held water (90.2%); 372 tires had larvae and the larval prevalence was 74.4%. The percentage of tires containing larvae on each sample date are reported in Table 2. Using odds ratio analysis and contingency chi-square tables, control tires were 113.8 times more likely to contain larvae than treated tires ($\chi^2 = 291$, $P < 0.001$) 14 days after treatment. Control tires were 66.8 times more likely to contain larvae than treated tires ($\chi^2 = 276$, $P < 0.001$) 28 days after treatment. Control tires were 17.0 times more likely to contain larvae than treated tires ($\chi^2 = 139$, $P < 0.001$) 65 days after treatment.

Table 2. Percentage of tires with mosquitoes in sites treated with VectoLex and untreated control.[1]

Section	Time 0	Day 14	Day 28	Day 65
1	80.7 (100)	2.9 (102)	2.9 (107)	2.9 (105)
2	89.8 (100)	6.7 (104)	1.0 (103)	1.1 (91)
3	83.6 (100)	4.9 (103)	6.1 (104)	15.4 (75)
4	88.9 (100)	7.7 (104)	1.0 (102)	12.7 (80)
5	69.6 (100)	4.8 (105)	1.0 (104)	8.6 (88)
Control	—	86.7 (60)	61.2 (103)	55.0 (100)

[1] The number of tires sampled in each section is given in parentheses.

DISCUSSION

VectoLex and Altosid were both effective for 1 month despite the fact that catch basins vary in their physical capacity and the amount of water they contain is influenced by neighborhood activities. Furthermore, lack of rainfall may increase oviposition in the remaining basins holding water, which in turn can influence the rate of depletion of the toxicant. In Table 1, note that when 10 of 26 Altosid-treated catch basins were positive 30 days after treatment, almost one half (20 of 46) of all catch basins sampled were dry. Similarly, when 11 of 28 VectoLex-treated catch basins were positive 35 days after treatment, 39% (18 of 46) of all catch basins sampled were dry. This study validated the application interval used by the CUEPP as well as the label for Altosid, which stated that a single briquet treats up to 100 ft^2 (9.29 m^2) or 75 gallons (285 liters) of water for 30 days. The label for VectoLex did not state the duration of activity nor did it specify a volume of water that could be treated by a given dose.

Our catch basin data are in agreement with those of Mulla et al. (1997) who reported that VectoLex was effective in domestic polluted waters for 4 wk, and Hougard (1990) reported that *B. sphaericus* strains 1593 and 2362 had residual effects of 3–4 wk in cesspits. In this study, VectoLex was effective for 44 days in one catch basin with frequent oviposition. Further studies must be conducted in order to determine how to maximize the duration of activity of VectoLex. We did not evaluate the maximum persistence of Altosid in this study but note that in a previous study a single briquet of Altosid was effective for as long as 50 days (Siegel and Novak 1997). In all likelihood a bit of a cushion exists for application of either product. If the maximum duration of activity is of interest, additional studies must be conducted that focus on the interval between 4 and 8 wk after treatment.

Assessing the efficacy of Altosid by larval collection may underestimate its activity, if the water brought back to the laboratory had a lower concen-

tration of Altosid than the catch basin, which in turn enabled successful adult emergence. Likewise, if Altosid degraded more rapidly in our rearing cups than it did in the catch basins, this would underestimate its effect because adults would successfully emerge. However, the larvae brought back to the laboratory were in the 4th instar, and in 4 cases pupae were recovered. We interpret this to mean that larvae were undergoing successful development in the catch basins, and therefore believe it is unlikely that the persistence of Altosid was underestimated. Our methodology may also have underestimated the efficacy of VectoLex because we did not evaluate delayed lethal effects such as death during pupation or adult emergence, nor did we evaluate sublethal effects. If efficacy was underestimated in this study, we believe that our error was equally distributed between the 2 treatments.

At current pricing levels, VectoLex enjoys a substantial advantage over Altosid briquets. This is balanced by differences in the ease of application. The VectoLex granules had to be distributed using a calibrated scoop, whereas the Altosid briquet was easily removed from its sheet. If a motor vehicle is the primary means of treatment, Altosid is currently easier to apply. However, many of the catch basins treated by the CUEPP are reached on foot, and in this situation no difference was found in ease of treatment. The decision to use one or the other product will involve balancing these respective advantages.

Treating a tire pile is challenging because of its spatial complexity as well as differences in toxicant penetration due to both stacking strategy and the method chosen to apply the toxicant. In a column, less than 2% of the granules may penetrate to the bottom row (Siegel et al. 1996). The stacking strategy used in a tire pile can also affect the penetration of the toxicant, with shingle-stacked tires the most difficult to treat because of the small openings. Application from a cherry picker, with the toxicant applied above the tire piles, is probably the most efficient (Novak et al. 1990). When a backpack sprayer is used by an applicator on foot, it may be necessary to direct the application vertically so granules can "rain" into stacked tires. This in turn decreases the efficiency of the application. Siegel et al. (1996) reported that in a large tire pile treated with a backpack applicator only 10–15% of the granules reached the inside of the tires.

The VectoLex label provided no guidance for its use in tire piles, because it listed a maximum rate per hectare based on a uniform water surface. In order to apply an efficacious dose of toxicant our focus was on our estimate of the number of tires in our study site. We knew that we had approximately 6,000 tires to treat and assumed that only 10% of the application would reach the target. Based on our previous research (Siegel et al. 1996) the desired dose was 376 mg (approximately 120–140 granules) per tire and in order to ensure delivery, we allocated 3.76 g per tire (22.6 kg total). This approach did not take into account volume differences between car and truck tires.

Although our dose per tire does not seem astronomically high, in order to achieve this goal, parts of the tire pile were treated with an application rate as high as 1,876.5 kg per hectare. This particular monitoring bucket was located next to a stack of truck tires that were treated by discharging the granules vertically so that they could "rain" in. In other areas within the stack, tires were piled in columns of 20, and a similar application method was used. Coverage within a pile is dependent on applicator experience combined with ease of access, application height, and the type of stacking used; no 2 piles are the same. All of these factors make it impossible to recommend a standard dose for a tire pile, but by focusing on the number of tires present we believe that control can be achieved.

Our application rate of 3.76 g/tire clearly suppressed *Ae. triseriatus* and *Culex* species larvae for 65 days. We believe that the duration of control would have been longer, but we had to terminate our study at this point. We note that even after 65 days, one section of tires was only 1.1% positive. This length of control exceeds that reported for *Bacillus thuringiensis* var. *israelensis* in tires (Novak et al. 1985) and is comparable to the duration reported by Siegel and Novak (1997). We note that Yap (1990) reported that a *B. sphaericus* dose of 10 ppm of was toxic to *Ae. triseriatus* for 9 months.

In conclusion, VectoLex CG was an effective larvicide when applied to catch basins and waste tires. We were able to replicate our previously reported data (Siegel and Novak 1997) in a large-scale study. Although the effectiveness of VectoLex was comparable to that of Altosid in catch basins, Altosid was easier to apply from vehicles. A tablet formulation of VectoLex would eliminate this advantage. Our study underscored the challenge of determining a realistic dose of VectoLex to treat tire dumps, when the current label recommendation is appropriate for sewage lagoons and drainage ditches. Further research is necessary to establish a label recommendation for tire treatments.

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